REMARKS

A Summary of the Invention

Broadly, the present invention concerns a method of determining the identity of one or more nucleotide basis at a plurality of specific positions in one or more nucleic acid molecules of interest. The method includes the step of treating a sample comprising the nucleic acid molecules of interest if the nucleic acid molecules are double stranded so as to obtained unpaired nucleotide bases spanning the specific positions. Alternatively, a sample of the nucleic acid of interest may be used directly if the nucleic acid molecules are single stranded. The method of the invention includes the step of contacting the sample with a plurality of different oligonucleotide primers. Each different oligonucleotide primer hybridizes under high stringency hyberization conditions to a corresponding different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the specific position of a nucleotide base to be identified with that oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the primer. Each different oligonucleotide primer comprises a corresponding different affinity moiety. The oligonucleotide primer comprising the affinity moiety is capable of hybridizing with a nucleic acid template and undergoing a nucleic acid template-dependent primer extension reaction with terminator of a terminator reagent. The affinity moiety permits affinity separation of the extended oligonucleotide primer from the terminator reagent. The method of the invention includes the further step of contacting the duplexes with a terminator reagent which includes four different terminators of a nucleic acid template dependent primer extension reaction. The terminator reagent is free of dATP, dCTP, dGTP, and dTTP. Each terminator comprises a different detectable label corresponding to the terminator. One of the terminators is complementary to a nucleotide base to be identified by each of the oligonucleotide primers. The contacting is carried out in a primer extension reaction

medium under conditions sufficient to permit a template dependent primer extension reaction, which incorporates the complimentary terminator onto the 3' end of each of the different oligonucleotide primers to thereby extend the 3' end of each of the primers by one terminator. The method of the invention further includes the step of affinity separating the respective extended oligonucleotide primers from the primer extension reaction medium by causing each of the extended oligonucleotide primers to contact an affinity group attached to a solid support. The affinity group is complementary to the affinity moiety incorporated in the oligonucleotide primer. Finally, the method of the invention includes the step of determining the presence and identity of the nucleotide base at each of the respective specific positions in the one or more nucleic acid molecules of interest by detecting the detectable label of the terminator incorporated at the 3' end of each of the affinity separated extended oligonucleotide primers.

B Summary of the Outstanding Office Action

Claims 64, 66, 67, and 60 through 70 inclusive were rejected in the Office Action of 26 November 2004 under 35 U.S.C. § 103(a) as unpatentable over European published patent application EP 0 412 883 A1 to Cohen *et al.* ("the Cohen *et al.* '883 published European application") or French patent 2,650,840 also to Cohen *et al.* ("the Cohen *et al.* '840 French patent"), each in view of international PCT published patent application WO 90/11372 to Davis *et al.* ("the Davis *et al.* '372 PCT published application").

It was noted in the Office Action that the Cohen *et al.* '883 published European application claimed priority to a French patent application 8910802, which issued as the '840 French patent. Since both the Cohen *et al.* '883 published European application and the Cohen *et al.* '840 French patent are in the French language and since an English translation of the '840 patent has been provided in the present case, only the '840 French patent will be referred to specifically in the discussion which follows.

It was asserted in the outstanding Office Action that the '840 French patent disclosed a method of determining the identity of one or more nucleotide bases in a nucleic acid molecule which involved contacting a single-stranded nucleic acid sample with an oligonucleotide primer to form a duplex between the primer and complementary target nucleic acids present in the sample, wherein the primer hybridized immediately 3' of the nucleotide to be determined. It was asserted that the method of the '840 French patent further included the step of contacting the duplexes with a solution containing four different terminators, each labeled with a different detectable moiety. The method of the '840 French patent assertedly further included the steps of extending the primer with the terminator and determining the identity of the incorporated terminator to determine the identity of the nucleotide base. It was conceded in the Office Action of 26 November 2004 that the Cohen *et al.* '840 French patent did not disclose performing the primer extension reaction using multiple primers, each comprising a different affinity moiety.

It was asserted in the Office Action of 26 November 2004 that the Davis *et al.* '372 PCT published application disclosed a method for determining the identity of one or more nucleotide bases in a nucleic acid molecule which comprised contacting a single-stranded nucleic acid molecule with an oligonucleotide primer to form a duplex between the primer and complementary target nucleic acids. It was asserted that the duplexes were contacted with a solution containing labeled dNTPs to extend the primer with the dNTPs, assertedly such that if the primer were perfectly complementary with the target nucleic acid, an extension product would be formed, but if the primer contained a mismatch at or near the 3' end of the primer, an extension product would not be formed. It was asserted in the outstanding Office Action that, in the method of the '372 PCT publication, the presence of an extension product was detected in order to determine the identity of a nucleotide base. In the Office Action, it was asserted that the '372 PCT publication disclosed that the identity of multiple nucleotides could be determined simultaneously by using a mixture of different oligonucleotides, in which each oligonucleotide

comprised a unique tail. It was asserted that, following the extension reaction, the primer extension/target nucleic acid complex was denatured and the primer extension product was hybridized to a solid support having bound thereto sequences complementary to the primer tail. It was asserted in the Office Action of 26 November 2004 that the unique tail allowed for the primers to be immobilized at specific locations on the support.

It was asserted in the Office Action of 26 November 2004 that it would have been obvious to have modified the method of the Cohen *et al.* '840 French patent so as to have used multiple primers, each having a different tail, and to have separated the primer extension products from the reaction medium by contacting the extension products with a solid support having immobilized thereon a capture probe complementary to the tail sequence, assertedly in order to accomplish objectives assertedly set forth in the Davis *et al.* '372 PCT published application.

Claim 68 was rejected in the Office Action of 26 November 2004 under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '883 published European application or the Cohen *et al.* '840 French patent, each in view of the Davis *et al.* '372 PCT published application and United States patent No. 5,332,666 to Prober *et al.* ("the Prober *et al.* '666 patent"). It was conceded in the outstanding Office Action that the hypothetical combination of the Cohen *et al.* '840 French patent and the Davis *et al.* '372 PCT published application proposed in the Office Action did not disclose using a terminator that comprised arabinoside triphosphate. It was asserted that the Prober *et al.* '666 patent disclosed that a terminator may contain an arabinose as the sugar group. It was asserted that in the Office Action of 26 November 2004 that it would have been obvious to one of ordinary skill in the art to have modified the method of the Cohen *et al.* '840 French patent so as to have a terminator comprising an arabinoside triphosphate.

Claim 71 was rejected in the outstanding Office Action under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '883 published European application or the Cohen *et al.* '840 French patent, each in view of the Davis *et al.* '372 PCT published application and United States patent No. 4,962,020 to Tabor *et al.* ("the Tabor *et al.* '020 patent"). In the Office Action it was conceded that the hypothetical combination of the Cohen *et al.* '840 French patent and the Davis *et al.* '372 PCT published application proposed in the Office Action did not disclose including pyrophosphatase in the primer extension medium. It was asserted that the Tabor *et al.* '020 patent disclosed including pyrophosphatase in the primer extension reactions to remove pyrophosphate which builds up in such reactions. The Tabor *et al.* '020 patent assertedly disclosed that, in the presence of pyrophosphate, DNA polymerase adds pyrophosphate to the 3' terminal nucleotide, assertedly causing release of dideoxynucleoside 5'-triphosphates, removing the block at the 3' terminus. It was asserted that in the Office Action of 26 November 2004 that it would have been obvious to one of ordinary skill in the art to have modified the method of the Cohen *et al.* '840 French patent so as to have included pyrophosphatase in the reaction medium assertedly to eliminate pyrophosphorolysis activity of DNA polymerase assertedly to reduce the probability that a labeled terminator would be removed and unlabeled dideoxynucleotides would be released into the reaction medium.

C Request for Reconsideration

Reconsideration of the subject application in light of the comments below is respectfully requested.

D The Rejections Under 35 U.S.C. § 103(a)

As discussed in the following subsections, the attorneys for the applicants submit that the Cohen *et al.* '840 French patent teaches directly against the hypothetical combination of the process for identifying a single base in a nucleic acid sequence of the '840 French patent with the method of the Davis *et al.* '372 PCT published application for testing a single sample of DNA

simultaneously for multiple alleles or for testing simultaneously at multiple loci for a single allele or multiple alleles proposed in each of the rejections 35 U.S.C. § 103(a) set out in the outstanding Office Action and that therefore the rejections are without merit and should be withdrawn.

D.1 The Cohen *et al.* '840 French Patent in View the Davis *et al.* '372 PCT Published Application

As disclosed at page 4, line 29 through page 5, line 19 of the Cohen *et al.* '840 French patent, the process of the patent for detecting a specific nucleotide base present on a nucleic acid sequence involved hybridizing the sequence in which the base to identified is located with a "trigger" nucleotide which hybridizes with its 3' end adjacent to the specific nucleotide base to be detected. Synthesis of the complementary strand of the resulting hybrid is initiated in the presence of a polymerase without 3'-to-5' exonuclease action and at least one modified nucleotide base capable of being incorporated into the extension product of the trigger nucleotide and of blocking further elongation of the extension product. The process of the Cohen *et al.* '840 French patent further involved detecting the incorporated blocking nucleotide base to identify the specific complementary nucleotide base located in the target nucleic acid sequence. According to page 5, lines 23 through 31 of the '840 French patent, the blocking nucleotide bases could be dideoxynucleotides marked with radioactive substances, enzymes, fluorescent or chemoluminescent chromophoric chemical products, or antibodies.

At page 6, lines 29 through 33 of the Cohen *et al.* '840 French patent, it was disclosed that a purported advantage of the process of the patent was that the process did not require immobilization of the nucleic acid on a membrane. As may be seen, for example, at page 1, lines 5 through 13, and page 2, lines 8 through 18 of the '840 French patent, in the context of the patent the term "nucleic acid" applies generally to each strand of hybridized DNA or RNA, including probes 150 nucleotides long and shorter probes. The necessity to immobilize nucleic acid on a membrane was specifically pointed out in the Cohen *et al.* '840 French patent to be a

disadvantage of the previously-known Southern blot technique and the method of United States patent No. 4,656,127 to Mundy *et al.* See page 3, lines 10 through 17 and page 4, lines 14 through 17 of the '840 French patent. It is submitted therefore that the Cohen *et al.* '840 French patent would have directly led persons skilled in the art away from any technique involving immobilization of nucleic acid on a membrane.

Moreover, it is submitted that the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application, proposed in the outstanding Office Action to be combined with the single-base-identification process of the Cohen *et al.* '840 French patent, would have been recognized by persons of ordinary skill in the art as just such a technique involving immobilization of nucleic acid on a membrane from which the '840 French patent taught away. The '372 PCT published application disclosed a technique for determining the existence or nonexistence of a test nucleotide on a strand of DNA which employed a polymerization agent capable of synthesizing an extension product if there were a match between the test nucleotide on the DNA strand and a nucleotide opposite on an extension primer, but not if there were a mismatch. According to page 5, line 19 through page 6, line 22 of the Davis *et al.* '372 PCT published application, a single sample of DNA could be tested simultaneously for multiple alleles at a single locus or for a single allele or multiple alleles at multiple loci by treating the DNA with a plurality of different oligonucleotide primers, each primer being complementary to a different allele and each having a unique "tail." The primers and the DNA are then subjected to conditions that would allow the primers and DNA to pair and extension products to form if there were a match between a test nucleotide and the opposite nucleotide on the primer, but not if there were a mismatch. It was disclosed at page 6, lines 7 through 22 of the '372 PCT published application the presence or absence of a particular extension product could be determined by applying the putative extension products to a substrate spotted at distinct locations with unique oligonucleotides complementary to each of the unique tails. According the

of the '372 PCT published application, if a particular extension product existed, it would attach to the substrate at only one location by way of hybridization of the unique tail to the complementary oligonucleotide found only at that location on the substrate. By detecting the presence of an extension product at a specific location on the substrate, the presence or absence of a specific allele in the test DNA could be determined.

It is submitted that, assuming for the sake of argument only that the hypothetical combination of the single-base-identification process of the Cohen *et al.* '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the outstanding Office Action would even have occurred to a person of ordinary skill on the art as of the effective date of the subject application, such a person would have recognized that the method of the Davis *et al.* '372 PCT published application involved immobilizing nucleic acid on a substrate and that the method would therefore have effectively shared the disadvantages of previously known methods requiring immobilizing nucleic acid on a membrane specifically pointed out in the '840 French patent It is submitted therefore that a person of ordinary skill in the art would not have attempted to combine the single-base-identification process of the Cohen *et al.* '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application in view of the teachings in the '840 French patent directly away from such the hypothetical combination.

For the reasons set forth above, it is submitted that the rejection in the Office Action of 26 November 2004 of claims 64, 66, 67, and 60 through 70 inclusive of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application was without justification and should be withdrawn.

D.2 The Cohen *et al.* '840 French Patent in View the Davis *et al.* '372 PCT Published Application and the Prober *et al.* '666 Patent

The Prober '666 patent in no way overcomes the teachings of the Cohen *et al.* '840 French patent against the hypothetical combination of the single-base-identification process of the '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the outstanding Office Action discussed in the preceding subsection and consequently the reasoning of the preceding subsection applies equally with respect to the rejection of claim 68 in the Office Action of 26 November 2004 under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Prober *et al.* '666 patent. It is submitted that the rejection in the Office Action of 26 November 2004 of claim 68 of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Prober *et al.* '666 patent was unjustified and should be withdrawn.

D.3 The Cohen *et al.* '840 French Patent in View the Davis *et al.* '372 PCT Published Application and the Tabor *et al.* '020 Patent

As in the case of the Prober *et al.* '666 patent discussed in the preceding subsection, the Tabor *et al.* '020 patent in no way overcomes the teachings of the Cohen *et al.* '840 French patent against the hypothetical combination of the single-base-identification process of the '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the Office Action of 26 November 2004 discussed above and consequently the reasoning of the preceding subsection applies equally with respect to the rejection of claim 71 in the outstanding Office Action under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Tabor *et al.* '020 patent. It is submitted that the

rejection in the Office Action of 26 November 2004 of claim 71 of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Tabor *et al.* '020 patent was unwarranted and should be withdrawn.

E Conclusion

For the reasons set forth above, it is submitted that the claims of the subject application as amended are patentable over the art of record considered alone or in any combination. Early allowance of the application is therefore earnestly solicited.

Respectfully submitted,

Attorneys for the Applicants

by: _____
J. David Ellett, Jr.
(Reg. No. 27,875)

Telephone No. (212) 813-1600